SEPARATION OF POLYPEPTIDE MONOMERS

RELATED APPLICATIONS

This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application No. 60/087,602 filed Jun. 1, 1998, the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a process for separating polypeptide monomers from dimers and/or other multimers using ion-exchange chromatography.

2. Description of Background and Related Art

Attempts to purify authentic, properly folded protein from recombinant hosts have been frustrated due to the tertiary structure of the molecule. In this regard, purification of the recombinantly produced molecule often yields a heterogeneous mixture that consists largely of inactive, misfolded, insoluble, and/or soluble dimers, multimers, and disulfide-linked aggregates. Other aberrant molecules, such as fragments, nicked, oxidized, and glycosylated forms, may also be present. Thus, purification is difficult and yields of the authentic monomer are often low. See, e.g., Elliott et al, *J. Protein Chem.,* 9: 95–104 (1990).

Different techniques have been used to correct these problems. For example, Chang and Swartz, *Protein Folding: in vivo and in vitro* (American Chemical Society, 1993), pp. 178–188 describe a method for solubilizing aggregated IGF-I produced in *E. coli*, using low concentrations of urea and dithiothreitol (DTT) in an alkaline buffer. U.S. Pat. No. 5,231,178 describes a method for the purification of correctly folded, monomeric IGF-I from *P. pastoris* using a combination of cation exchange, hydrophobic interaction, and gel filtration chromatography. WO 96/40776 describes a method for producing authentic properly folded IGF from yeast using a first cation exchange chromatography with the yeast cell medium, denaturing and chromatography, and performing reverse phase high performance liquid chromatography.

Separation of protein and peptide monomers from their dimers, tetramers, and multimers presents a serious challenge to the separations scientist. Size-exclusion chromatography (SEC) and Tangential-Flow Filtration (TFF) (U.S. Pat. Nos. 5,256,294 and 5,490,937) have been used for separating monomers from aggregates but have limitations. SEC can separate monomers from multimers, and in some cases monomers from dimers. The main limitations of SEC are 1) limited load volumes (typically 5% of the bed volume) requiring large columns or multiple cycles, 2) and load protein concentration (low concentration feed stocks require pre-concentration or multiple cycles on the column. Higher protein concentrations can be more viscous, thereby reducing the efficiency of the separation). Historically TFF can separate protein multimers that are ten-fold larger than the monomer. U.S. Pat. No. 5,256,294.

U.S. Pat. Nos. 4,228,154 and 5,250,663 disclose separations of albumin from mixtures. U.S. Pat. No. 4,228,154 describes use of both cation-exchange and anion-exchange chromatography steps for the purification, without separation of monomer from multimers.

There is a need for separating monomers from dimers and multimers that is satisfactory, requires the use of only one ion-exchange step, and does not have the limitations of SEC or TFF.

SUMMARY OF THE INVENTION

Accordingly, this invention provides a method for separating a polypeptide monomer from a mixture comprising dimers and/or multimers, wherein the method comprises applying the mixture to either a cation-exchange or an anion-exchange chromatography resin in a buffer, wherein if the resin is cation-exchange, the pH of the buffer is about 4–7, and wherein if the resin is anion-exchange, the pH of the buffer is about 6–9, and eluting the mixture at a gradient of about 0–1 M of an elution salt, wherein the monomer is separated from the dimers and/or multimers present in the mixture.

In this study it is demonstrated that ion-exchange chromatography—either anion or cation—is an effective means to separate protein or polypeptide monomers from their dimers and/or multimers. Separations are performed using either step or linear gradient elution. Ion exchange has several advantages over the SEC and TFF methods described above. First, separation is independent of polypeptide concentration in the load and therefore no pre-concentration is required. Second, resins can be loaded to greater than 30 mg polypeptide/mL resin and still achieve excellent separations. Third, ion-exchange resins are inexpensive and easy to use. Typical separations achieve enrichment of monomer to greater than 99.5% purity and yields in excess of 90%.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A and **1**B show separation of U266 IgE monomer from dimers and multimers on a RESOURCE Q™ anion-exchange column. The column was equilibrated in 25 mM Tris/pH 8, and eluted with a gradient from 0 to 0.5 M sodium chloride over 10 column volumes. FIG. **1**A is full-scale; FIG. **1**B is a close-up view to show the dimers and multimers.

FIGS. **2**A**1**, **2**A**2**, **2**B, and **2**C show separation of anti-IgE monoclonal antibody monomer from dimers and multimers. FIGS. **2**A**1** and **2**A**2** were run on a RESOURCE Q™ anion-exchange column. FIG. **2**A**1** is full-scale; FIG. **2**A**2** is a close-up view to show the dimers and multimers. FIG. **2**B is a run on Q-SEPHAROSE FAST-FLOW™ resin. FIG. **2**C is a plot of monomer and dimer/multimer observed in fractions, where the open dots are monomer and the solid dots are dimer. The monomer and dimer/multimer were determined using a SUPERDEX 200 HR™ 10/30 analytical size-exclusion column (Pharmacia Biotech). In all cases the columns were equilibrated in 25 mM Tris/pH 8. The gradient used in the FIG. **2**A panels was 0 to 0.5 M sodium chloride over 40 column volumes. The gradient used for FIG. **2**B (Q-SEPHAROSE FAST-FLOW™) was 0.05 to 0.2 M NaCl over 10 column volumes.

FIGS. **3**A–C show separation of BSA monomer and dimer on a RESOURCE Q™ anion-exchange column at pH 8. The column was equilibrated in 25 mM Tris/pH 8, and eluted with a gradient from 0.125 to 0.275 M sodium chloride over 40 column volumes. FIG. **3**A is purified monomer, FIG. **3**B is purified dimer, and FIG. **3**C is a commercial preparation of BSA (Bayer) that contains both monomer and dimer.

FIGS. **4**A–C show separation of BSA monomer and dimer on a RESOURCE Q™ anion-exchange column at pH 6. The column was equilibrated in 20 mM sodium phosphate/pH 6, and eluted with a linear gradient from 0 to 0.5 M sodium chloride over 10 column volumes. FIG. **4**A is purified monomer, FIG. **4**B is purified dimer, and FIG. **4**C is a commercial preparation of BSA (Bayer) that contains both monomer and dimer.